

Ant – Plant Interactions

Edited by

CAMILLA R. HUXLEY

Department of Plant Sciences, University of Oxford

and

DAVID F. CUTLER

Royal Botanic Gardens, Kew, Richmond, Surrey

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Variation in the attractiveness of lycaenid butterfly larvae to ants

N. E. Pierce, D. R. Nash, M. Baylis, and E. R. Carper

Introduction

Although this volume is primarily concerned with ant–plant interactions, the association between lycaenid butterflies and ants brings an extra dimension to bear on such relationships by introducing an additional trophic level, or, to be slightly more accurate, an additional organism that links two levels. As has been argued by May (1973), the length of a food web can be related to its stability. Communities with many species are likely to be less stable following perturbation than those with fewer species, and variations are amplified as they propagate through the system when the food web is more complex (Pimm 1982). Discussions of stability and complexity have largely focused on food webs whose third-level trophic interactions are essentially antagonistic: for example, the role of predation and parasitism in structuring herbivorous insect communities has been considered at length (Price *et al.* 1980; Strong *et al.* 1984). Somewhat less attention has been paid to how mutualisms may influence community structure and diversity (Addicott 1986; Cushman and Addicott, Chapter 8, this volume).

Mutualism can be defined as an interaction in which the genetic fitness of each participant is increased by the action of its partner. One of the intriguing aspects of mutualisms is that they vary widely, from facultative, indirect and/or diffuse relationships, to highly co-evolved, complex, and often obligate associations. This same variability makes it difficult to derive abstract models of mutualism that are generally applicable. The range in the strength of interactions suggests that the relationship between mutualistic partners is often fragile, subject to environmental fluctuations in which alternative lifestyles may well be more attractive.

The association between lycaenid butterflies and ants represents an ideal system for research on mutualism. The family Lycaenidae is characterized by extreme variety in life histories, ranging from facultative and obligate mutualisms to parasitism. Lycaenids seem to specialize in complex interactions involving other groups of insects, usually ants. Thus, the larvae of some species are carried by attendant ants into the nest where they feed on the ant brood; the males of others use ants as cues in finding pupae that are about to

eclose and then engage in frenzied battles for access to mates; the larvae of others are entirely carnivorous, specializing on species of soldier-forming aphids whose soldiers are relatively ineffective against the marauding caterpillars (Cottrell 1984; Pierce 1987).

In a great many lycaenids, larval-ant relationships appear to be mutualistic in ways that are analogous to homopteran-ant associations. While comparisons are useful, it is worth keeping in mind that these interactions differ in at least one essential feature: the secretions produced by lycaenid caterpillars for their attendant ants are derived from specialized glands, and are not a modified excretion, as is the honeydew of homopterans. Lycaenid secretions are expensive to produce, and any caterpillar that achieves the maximum benefit for the minimum cost will be at a distinct advantage relative to its conspecifics. Thus, in some species, selection has favoured the evolution of secretions that mimic ant recognition signals (Henning 1983; Thomas *et al.* 1989).

In this study we investigate how variation in three features of the environment shapes the strength of the relationship between a lycaenid butterfly and ants. We have chosen a complex mutualism that is obligate for one of the partners because it allows us to measure some of the costs and benefits of the interaction in a straightforward manner, and because it enables us to tease apart contributing variables that might be less obvious or more difficult to quantify in a more diffuse interaction. Manipulating various aspects of an obligate mutualism and observing the consequences also provides us with insights as to the mechanisms by which obligate mutualisms are able to persist through such environmental fluctuations. In particular, we describe how variability in the host plants, the attendant ants, and the social organization of the butterflies can affect the mutualism. Having identified some of the ecological hurdles that the butterflies face, we then discuss ways in which they are adapted to survive these challenges.

The system we have been studying is that of the Australian lycaenid, *Jalmenus evagoras* Donovan, the larvae of which feed on species of *Acacia*, and both the larvae and pupae associate with several species of ants in the genus *Iridomyrmex*. While predators and parasitoids of *J. evagoras* are patchily distributed, and attendant ants are more effective against some enemies than against others, the net effect of ant removal is essentially the same: larvae and pupae of this butterfly cannot survive without attendant ants. The colonies that tend larvae and pupae of *J. evagoras* harvest secretions that contain both sugars and concentrated free amino acids; these nutritious rewards result in higher rates of colony growth (Pierce 1983; Nash 1989). Colonies of attendant ant species have alternative food sources and may be found without butterfly associates (Smiley *et al.* 1988). Thus, the relationship appears to be obligate from the point of view of the lycaenids, whereas the attendant ant species receive a net benefit from the association, but can survive without the butterflies.

The natural history of *Jalmenus evagoras*

Jalmenus evagoras has a widespread distribution within Australia, ranging from Melbourne, Victoria in the south to Gladstone, Queensland in the north, and occurring both along the coast and inland on the ranges (Common and Waterhouse 1981). Larvae feed on the foliage of about twenty different species of *Acacia*. Females lay eggs in clusters beneath loose bark, and both the larvae and pupae form large and visible aggregations that are attended by myriad, small, black ants. To date, we have observed at least five different species of *Iridomyrmex* ants tending the juveniles in the field, although two of these are particularly common, a small black one in the *vicinus* Clarke species group (here called *I. vicinus* for convenience), and a larger black one in the *anceps* Roger species group (referred to as *I. anceps*). All the ants that tend *J. evagoras* are closely related, and the majority of other *Iridomyrmex* species found in the same regions are predators of the lycaenid caterpillars.

Despite the broad geographical range of this species, populations of *J. evagoras* are discrete and highly localized, probably as a result of their dependence upon attendant ant colonies and suitable host plants (Smiley *et al.* 1988). Thus, at one field site we were able to observe 74 out of 80 marked, individual butterflies almost daily for their entire estimated lifespans (about three days for females and seven days for males) (Elgar and Pierce 1988). Males of *J. evagoras* tend to eclose several days earlier than females, and the effective sex ratio in the field is usually strongly male biased. Adult males search for mates by regularly investigating trees containing juveniles of the species, and they use ants as cues during this searching process. They hover around clusters of pupae, occasionally appearing to tap them with their antennae. When a pupa is about to eclose, it is not uncommon to find a dozen males clustered around it: after a fierce struggle among the males, the female is mated before she has even expanded her wings.

Both the larvae and pupae of *J. evagoras* secrete food for ants from specialized exocrine glands, and in return the ants protect them against parasites and predators. Five field experiments conducted over three years have demonstrated that predation and parasitism, largely by invertebrates, is so intense that the larvae and pupae of *J. evagoras* cannot survive in the field without attendant ants, although they can be successfully reared without them in the laboratory (Pierce *et al.* 1987; D. R. Nash and M. Baylis, unpublished work). Not surprisingly, female butterflies use ants as cues when they lay eggs (Pierce and Elgar 1985). Moreover, when given a choice, ovipositing females can distinguish between different kinds of ants, and respond preferentially to the appropriate species (N. E. Pierce, unpublished work). In addition to guarding juveniles, attendant ants can shorten larval duration by as much as 14 per cent, thereby reducing the time that larvae are exposed to predators and parasitoids (Pierce *et al.* 1987).

The cost of associating with ants is expressed as a reduction in adult weight

and size, presumably due to the material given up to ants (Baylis 1989). For example, in one experiment, females that were tended by ants pupated at a weight which was approximately 20 per cent lighter than their untended counterparts (Pierce *et al.* 1987). This difference in size definitely represents a cost: relative size and weight are important components of both lifetime mating success in males, and fecundity in females (Elgar and Pierce 1988).

Attendant ants benefit nutritionally from their association with *J. evagoras*. In an experiment designed to examine the effect of larval secretions on the growth rate of colonies of *Iridomyrmex vicinus*, we found that the size of egg masses laid by queens of colonies which foraged on an artificial diet supplemented with secretions from five caterpillars was larger than that laid by queens of colonies which were supplemented with secretions from only one caterpillar. Both were larger than egg masses laid by queens whose colonies foraged on an artificial diet alone. Colony growth rates, as measured by the relative increase in numbers of larvae, pupae, and workers over a three-month period were higher for colonies fed artificial diet supplemented with secretions from either one or five caterpillars, than for colonies fed the artificial diet alone (Nash 1989).

The effect of host plant quality on larval attractiveness to ants

In 1878, Edwards commented upon the larval secretions of the North American lycaenid, *Celastrina (Lycaena) pseudargiolus*, 'it is probable that the quality of this . . . secreted fluid . . . and perhaps its attractiveness depends on the nature of the food plant' (p. 8). Since the food rewards that lycaenid larvae provide for their attendants are derived from their host plants, it is reasonable to suspect that variation in host plant quality might alter the quality of the rewards and hence the number of attendant ants (see Chapter 9). As the secretions of some lycaenids are known to be rich in proteins as well as carbohydrates, the protein concentration of the host plant may be of particular importance to the relationship. For example, the Lycaenidae as a whole are well known for their predilection for feeding on flowers, seed pods, and terminal foliage (all the protein rich parts of host plants) (Mattson 1980; Robbins and Aiello 1982). The larvae of *Glaucopsyche lygdamus* Doubleday attract more attendant ants when feeding on the seed pods of their host plant, *Lupinus floribundus* Greene, than they do when feeding on other parts of the plant, perhaps because the seed pods represent higher quality food (Pierce and Eastal 1986).

We investigated this question experimentally by manipulating the quality of the host plants consumed by larvae of *J. evagoras*, and observing the subsequent effect on ant attendance and larval survivorship. Young, potted food plants of *Acacia decurrens* Willd. were either given water containing a nitrogenous fertilizer, or were given water alone. Fertilized plants had a higher nitrogen content than their unfertilized counterparts. Under natural,

field conditions, fifth instar larvae of *J. evagoras* feeding on fertilized plants attracted a significantly larger number of attendant ants (mean \pm s.e.; 7.9 ± 0.8) than those feeding on unfertilized plants (6.2 ± 0.7). In the absence of caterpillars, ants were not differentially attracted to either plant type. Not only did caterpillars on fertilized plants attract a larger ant guard, but they also survived better in the field over a ten-day period than did larvae on unfertilized plants (Fig. 11.1). However, larvae reared in a screened bush house in the absence of ants and predators survived equally on fertilized and unfertilized plants. We concluded from this that larvae on fertilized plants survived better than their counterparts on unfertilized plants by attracting a larger ant guard (Baylis and Pierce 1991). Not surprisingly, we also found that females of *J. evagoras* preferred to lay egg batches on fertilized, rather than unfertilized plants (Baylis and Pierce 1991).

The effect of host plant quality on ant attendance and subsequent larval survival was both strong and immediate. Considering the importance of attendant ants to the survival of the larvae, we might have expected selection to favour larvae whose secretions were more constant and predictable despite fluctuations in host plant quality. However, the extreme sensitivity of the female butterflies to the condition of prospective host plants indicates that the butterflies do indeed possess a powerful mechanism for responding to this level of variation in their environment.

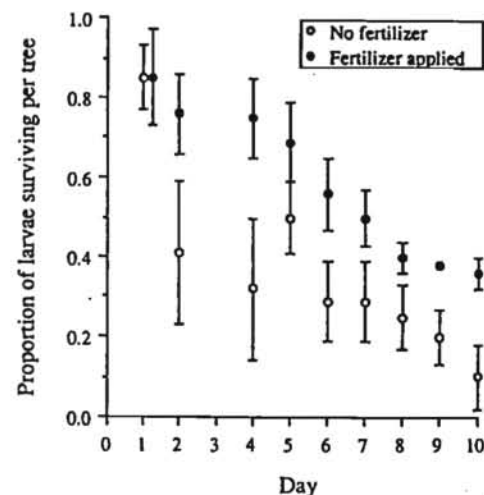


Fig. 11.1. Survival of larvae of *Jalmenus evagoras* as a function of host-plant quality. Points represent means, and error bars the standard errors of the proportion of larvae surviving on each of three plants per treatment (data for only one site shown; two-way ANOVA of treatment and site on proportion surviving per plant: day six, variance ratio = 4.40, d.f. 1,8; $P = 0.07$; day seven, variance ratio = 4.00, d.f. 1,8; $P = 0.08$). (Redrawn from Baylis and Pierce 1991.)

The effect that host plant quality can have on lycaenid-ant interactions is reflected in a more evolutionary sense by a comparison of the host plant usage of species that do and do not associate with ants. A survey of 297 species of Lycaenidae revealed that ant association is strongly correlated with the consumption of protein-rich, nitrogen-fixing plants. Thus the larvae of lycaenid species that feed on legumes are much more likely to associate with ants than the larvae of lycaenids that feed on other kinds of plants. Similarly, of the larvae of lycaenids that feed on non-leguminous plants, those on nitrogen-fixing plants are more likely to be ant-tended than those species that feed on non-nitrogen fixing plants (Pierce 1985).

The effect of ant nutrition and foraging behaviour on levels of tending

Variation in ant attendance of individual lycaenid larvae depends in part upon the spatial arrangement of each partner. Since ants that tend larvae are rewarded by food secretions, foraging theory would predict that the time spent by ants tending lycaenids, and hence the rates of ant traffic to and from those lycaenids, will vary as a function of foraging distance. We found this to be the case. Field experiments using larvae on potted host plants, placed at different distances from a central ant nest, showed that larvae placed at greater distances elicited lower flow rates of ants, and were more likely to be preyed upon than larvae placed closer to the ant nest (Carper 1989; Fig. 11.2).

In addition to spatial effects, colony size may be an important determinant in the number of ants available to tend larvae and pupae. Laboratory experiments demonstrated that colony size had a strong effect upon levels of attendance of larvae (Nash 1989). Presumably for young colonies, colony size is often correlated to colony age.

Finally, the nutritional state of the attendant ant colony can influence the attractiveness of individual larvae and pupae in complex ways. Some of our field work has focused on the behaviour of individual ant colonies as a function of their overall diet. In order to do this, we observed ant colonies in the field that were engaged in tending larvae and pupae of *J. evagoras*, and we recorded whether these colonies were also tending homopterans. As a rough measure of how much food each colony was harvesting from each food source, we scored the number of workers foraging on homopterans and the number foraging on larvae and pupae of *J. evagoras*, and in cases where a resource had more than thirty attendant ants, we measured the rate of flow of ants travelling to and from the resource. We then laid out a grid of one meter squares covering the area occupied by the ant colony, baited the vertices of the grid with sugar and canned tuna baits, and scored the number of ants foraging from these baits. We found that colonies that were predominantly tending larvae and pupae of *J. evagoras* sent out more workers to sugar than to tuna baits, whereas colonies that were foraging pre-

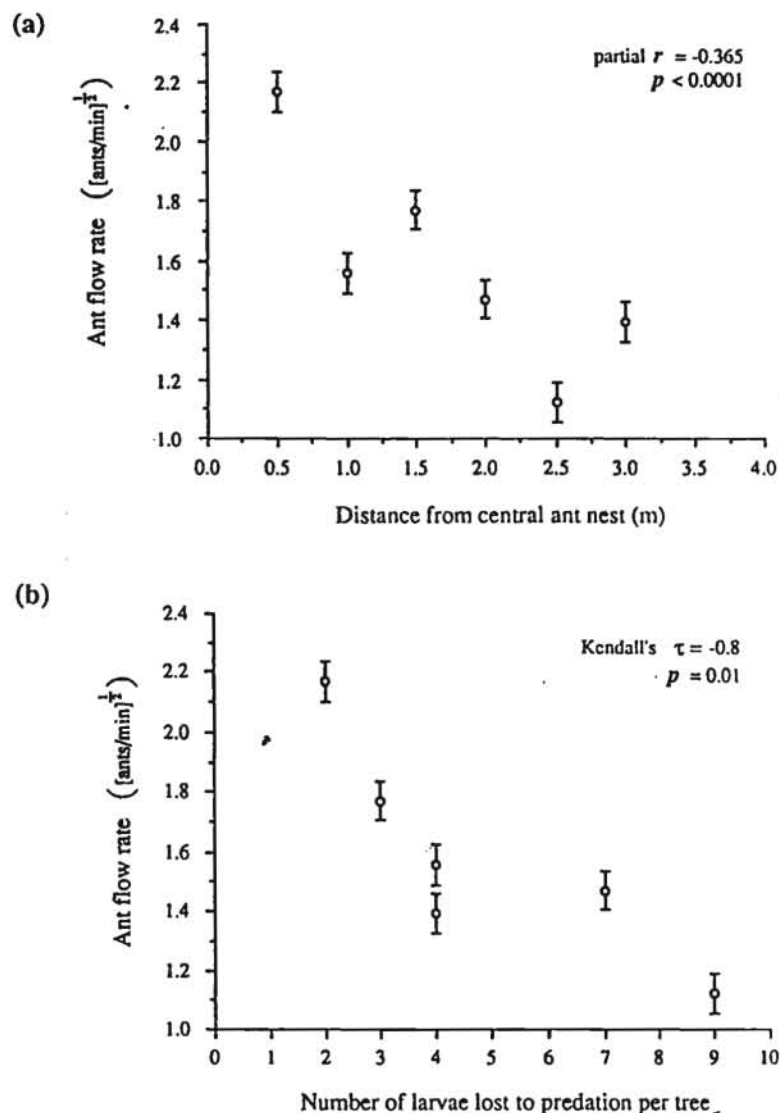


Fig. 11.2. The correlations between distance, flow rate of ants, and survival of larvae of *Jalmenus evagoras*. (Redrawn from Carper 1989.) (a) Variation in flow rates of ants to and from trees bearing *J. evagoras* as a function of ant foraging distance. Ant flow rate (transformed as the square root of the numbers of ants running to and from a tree per minute) was measured for potted host plants of *Acacia decurrens* containing six fifth-instar larvae of *J. evagoras* and placed at different distances from a nest of the ant, *Iridomyrmex anceps*. (b) Predation as a function of flow rates of *Iridomyrmex anceps* ants to and from *Acacia decurrens* trees bearing *Jalmenus evagoras*. The total number of larvae of *J. evagoras* lost by the trees described above over a two-week period are plotted as a function of ant flow rate to each tree. Whenever a larva disappeared, it was replaced with another. The total number of larvae lost per tree was negatively correlated with the mean ant flow rate to that tree.

dominantly from homopterans were more attracted to the tuna baits (Nash 1989; Fig. 11.3).

From these observations we tentatively conclude that the nutritional needs of the colony as a whole can best be met by feeding on both carbohydrates (homopteran honeydew and sugar) and proteins (lycaenid larvae secretions and tuna fish) (Brian 1973), and that the colonies we examined appeared to be compensating for a preponderance of one or the other of these nutrients in order to achieve a balanced diet. However, it is possible that the secretions of lycaenid larvae contain nutrients other than proteins that are important for colony growth, and that these nutrients are analogous in some way to nutrients provided by the canned tuna baits.

While this experiment did not address individual attractiveness of larvae and pupae, the results have clear implications for an important source of variation: the nutritional state of an ant colony (in which the brood in the nest can be regarded as a collective stomach) may influence the relative attractiveness of individual lycaenid larvae to potential attendant ants if there is varia-

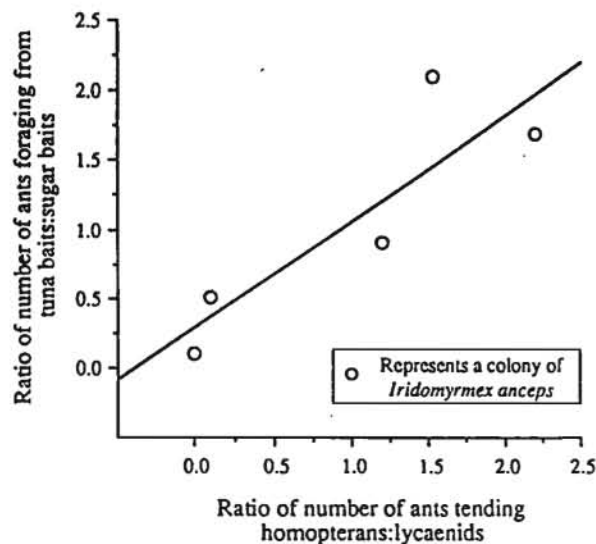


Fig. 11.3. Ant-foraging preferences as a function of the availability of alternative food resources. The five points represent five colonies of the ant *Iridomyrmex anceps* mapped at a field site at Cloud's Creek, New South Wales, Australia. The ratio of workers foraging from homopteran honeydew and from lycaenid larvae secretions is plotted against the ratio of the number of ants from the same colony that were sent to sugar and tuna fish baits. When colonies were foraging primarily on homopteran honeydew, they preferred canned tuna baits to sugar ones; however when they were foraging primarily on the secretions of the lycaenid, *J. evagoras*, they preferred sugar baits to canned tuna ones ($y = 0.81x + 0.246$; $r^2 = 0.824$; $P < 0.05$). (Redrawn from Nash 1989.)

tion in the nutritional composition of their secretions. Lycaenids such as *J. evagoras* whose juveniles rely upon ants for survival are in competition with the other food sources available to their attendant ants, and alternative food sources may thus exert profound indirect effects on the survival of the butterflies. Presumably, there is strong selection for lycaenids to secrete nutritional rewards that are somehow limiting in their attendant ant's diet, and therefore valuable. There may also be selection on the lycaenids to produce a complete diet for the ants (see Chapter 18) so that they do not need to spend time elsewhere. It might also be interesting to investigate whether colonies that are in the process of producing alates have different dietary requirements than those producing workers, and whether these needs are reflected in the numbers of workers attracted to lycaenid larvae and pupae (Brian 1973).

Cumulatively, these results suggest that the characteristics that make an ant a valuable mutualistic partner are not only interspecific differences such as the aggressiveness, size, and/or abundance of available workers. In addition, variables such as colony size, age, alternative food sources, growth rate, and/or ratio of foragers to brood may all contribute to the level of attendance by ants. It is reasonable to conclude that populations of obligate mutualists such as *J. evagoras* are constrained not only spatially by the distribution of appropriate host plants and attendant ants, but also temporally by the phenology and growth characteristics of both host plants and attendant ant colonies, and in a more indirect way by the spatial and temporal distribution of alternative food sources such as homopterans. Although we know that females of *J. evagoras* can detect the presence or absence of appropriate ant associates and use them as cues in oviposition, we would predict from these results that they must also be sensitive to differences in ant densities and perhaps to the distribution of other trophobionts.

The effect of conspecifics on individual variation in larval attractiveness

As described earlier, the larvae and pupae of *J. evagoras* aggregate, and this poses some interesting questions about individual variation in attractiveness to ants. If a threshold number of ants is necessary to protect the larvae and pupae, then aggregating may be one mechanism by which *J. evagoras* could simultaneously increase its collective defence and decrease the amount of food that each individual would need to produce to attract that defence. For example, our field observations showed that first-instar larvae could conceivably gain more ants by joining a group of any size than by remaining alone, and second or third instars could have a higher number of attendant ants by joining a mean sized group of about four larvae (Pierce *et al.* 1987).

In order to examine whether larvae selectively form groups in response to ant densities, we investigated the decisions made by final-instar larvae which

were about to pupate. We first determined that aggregations of pupae are indeed tended by more ants per pupa than equivalent numbers of pupae placed a similar mean distance from a central ant-nest, but in dispersed rather than clumped configurations (Carper 1989). Thus, a final-instar larva can increase its level of ant attendance as a pupa by pupating beside another individual rather than pupating alone. We then compared the pupation choices made by larvae on potted host plants where larval and/or pupal number was kept constant, but the flow rates of ants foraging to and from the plants varied as a function of distance. We found that larvae were extremely sensitive to the flow rates of ants on plants and adjusted their behaviour accordingly. Given a choice between pupating beside another individual or pupating alone, a larva was more likely to join a cluster when the mean ant flow rate to the tree was relatively low (about three ants per minute), whereas it pupated by itself when the mean flow rate was high (about six ants per minute) (Table 11.1). Similarly, a larva faced with the choice of pupating with a smaller or larger cluster of pupae joined the larger cluster when ant flow rates were low, but joined the smaller of the two clusters when ant flow rates were high (Carper 1989).

Table 11.1. Pupation decisions of larvae on trees with differing densities of attendant ants.

	Low ant flow rate	High ant flow rate
Pupate alone	0	4
Pupate in a group	15	7

When the ant flow rate to a tree was low (< 3.18 ants/min, the mean ant flow rate), a larva was more likely to pupate beside other pupae. When the ant flow rate was high (> 3.18 ants/min), a larva was more likely to pupate alone (Chi-square = 3.96 with continuity correction; $P = 0.047$; $N = 26$) (from Carper 1989).

In the field, it is not uncommon to observe that small larvae have pupated near the base of a host plant, directly in the trail of ants leading to large aggregations of pupae. Presumably these individuals receive a benefit from pupating in a location with a higher ant density than they might have been able to attract on their own. However, it would also appear that these larvae are simply making the best of a bad job. There are likely to be benefits gained by pupating at a higher position in a tree, perhaps related to a tradeoff in the costs and benefits of ant attendance, or perhaps to other factors such as the ease of discovery upon eclosion by potential mates. We found that large larvae which attracted many ants were more likely to pupate in a relatively

high position on a host plant than their smaller, less attractive counterparts (Carper 1989).

These complex behavioural responses suggest equally complex interactions between ant attendance and social context for the juveniles of *J. evagoras*. Consider the following possibility. The cost to larvae of ant attendance is pupating at a smaller size. If individual larvae and pupae vary in their attractiveness to ants, then there may be a kind of auto-mimicry taking place within aggregations of larvae and/or pupae whereby certain 'defecting' individuals in the population take advantage of 'co-operating' individuals. Defectors are those larvae and/or pupae that aggregate with their co-operative counterparts and enjoy the benefits of ant attendance, but do not secrete food in return and hence do not pay the costs. Such defection might be less likely if co-operative individuals aggregated preferentially with their kin. Nevertheless, selection could still favour an individual that adopted a strategy that involved not secreting, but aggregating with non-kin. This could result in a mixed ESS (evolutionary stable strategy) in which the ratio of defecting to co-operating individuals would be governed, in a frequency-dependent fashion, by the effectiveness of the ant guard and the predator and parasitoid pressure of the environment. Of course, this scenario would sound less far-fetched and more interesting if we could establish whether the ants can respond directly to the rates of secretion of caterpillars, and whether attractiveness to ants is a genetically variable trait in *J. evagoras*. This is work that is currently in progress.

In summary, the aggregation behaviour of larvae and pupae of *J. evagoras* allows the larvae to exert some influence over the level of ant attendance on the particular tree upon which they find themselves. Thus, if ant densities are low, they can join together to increase their per capita tending levels, whereas if ant densities are high, they can spread out, perhaps thereby reducing some of the costs.

Conclusions

The experiments described here illustrate how variation in three aspects of the interaction between lycaenid butterflies, their host plants, their attendant ants, and their predators and parasitoids can have ramifications at other levels in the system. Our analysis has taken the perspective of the butterfly. Since larval survival is correlated with the level of ant attendance, any perturbation that subsequently alters the level of attendance provides us with a powerful bioassay.

In each case, the butterflies described in this study are buffered against variability in their environment by their behavioural flexibility. This complexity is partly the result of the holometabolous life history of butterflies, in which the constraints of the relatively sessile larval stage are compensated for by the free-flying, and hence dispersive, adult stage. Thus, we

determined that if one alters the quality of the host plant, the level of ant attendance and hence larval survival also changes. The primary mechanism by which the butterflies appear to cope with this variation is through their selection of host plant. Females are extremely sensitive to the quality of their prospective host plants.

Similarly, if one alters the colony size, foraging distance, or nutritional state of the attendant ant colony, one may also affect the level of ant attendance and larval survival. Again, the mechanism that enables the butterfly to have some control over this variability is in its choice of oviposition sites: females can distinguish between different ant species and use ants as cues in laying eggs. We would further predict that females may respond to ant density and the distribution of alternative food sources (such as homopterans) when ovipositing.

Finally, the social behaviour of the larvae and the pupae also appears to affect levels of ant attendance in complex ways. Variation in the host plant quality and attendant ant colonies may affect the butterfly's overall distribution and clumping patterns, since females are more likely to lay eggs on high-quality host plants that also contain ants. However, even when a larva hatches out upon a particular host plant, its propensity to aggregate with conspecifics can affect its numbers of attendant ants. Joining or not joining a group is an additional fine-tuning mechanism by which a larva can adjust the level of ant attendance.

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